CHYTRIDS IN EGYPT: I - SAPROPHYTIC SPECIES OF THE CLADOCHYTRIACEAE FROM WATER STREAMS

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ABSTRACT - Eighteen zoosporic, polycentric members of saprophytic Cladochytridiaceous fungi belonging to four genera were isolated from water streams in El-Minia Governorate during autumn, winter and spring of 1987 and 1988 on cellulose-rich substrata (sterile baits of onion skin and corn husks). These fungal species flourished and developed within two or three weeks of incubation (21-23°C). Among the species isolated, seven are recorded for the first time in Egypt: *Cladochytrium taianum* Shen & Siang, *Megachytrium westonii* Sparrow, *Nowakowskiella crassa* Karling, *N. macrospora* Karling, *N. pitcairnensis* Karling, *Septochytrium macrosporum* Karling and *S. marylandicum* Karling.

RÉSUMÉ - Dix-huit espèces de Cladochytridiacées saprophytes (4 genres) ont été isolées de rivières du governorat d'El-Minia, en automne, hiver et printemps 1987 et 1988, sur pièges riches en cellulose (pelures d'oignons, téguments de céréales stériles). Ces champignons se développent en 2 ou 3 semaines d'incubation à 21-23°C. Parmi ces espèces, 7 sont nouvelles pour l'Egypte: *Cladochytrium taianum* Shen & Siang, *Megachytrium westonii* Sparrow, *Nowakowskiella crassa* Karling, *N. macrospora* Karling, *N. pitcairnensis* Karling, *Septochytrium macrosporum* Karling et *S. marylandicum* Karling.

INTRODUCTION

Cladochytriaceae Schroeter are characterized by a polycentric rhizomycelium which consists of fin or relatively coarse tenuous filaments, intercalary swellings, rhizoids, sporangia and resting spores. In *Cladochytrium* Nowakowski and other genera of this family the sporangia are inoperculate, while in *Nowakowskiella* Schroeter and *Septochytrium* Berdan they are operculate. There are approximatly 36 fairly well known species of this family.

Since Sparrow's monograph (1960) chytrids are commonly recognized in the rank of the order Chytridiales (Sparrow, 1973; Batko, 1975; Karling, 1977, 1987; Gupta & Mehrotra, 1989). Barr (1980) studying the ultrastructure of zoospores of Chytridiales divided this order into two emended orders Chytridiales and Spizellomycetales. Dogma (1973) had proposed the "cladochytrioid alliance" for this group of chytrids.

So far these fungi have received little attention in Egypt. Gaertner (1954) found three species belonging to this family. Karling (1976), reported four members in the African soil collections. Recently, Elnaghy et al. (1985 a, b; 1987) identified eleven species belonging to the Cladochytriaceae.

Cellulose-containing substrata were used in this study for isolation and subculturing the zoosporic fungi belonging to Cladochytriaceae.

MATERIALS AND METHODS

As recommended by Willoughby (1961) and Sparrow (1968), samples were collected from shallow water where soil is washed by waves near the banks. Samples were collected from different canals located in El-Minia Governorate, as shown in Fig. 1, from Deir-Mawas, Mallawi, Abo-Qurqas, El-Minia, Samalout, Mattai, Beni-Mazar and Maghaga, monthly during autumn, winter and spring of 1987 and 1988 which are the best seasons for multiplication of the saprophytic zoosporic fungi (Sparrow, 1960). Immediately after collection, 20ml aliquots of water samples were incubated at room temperature (20-23°C), with a cellulose-containing substrate (bits of sterilized onion skin scales, cellophane, bromegrasse leaves and corn husks) in sterile 9cm diam. petri dishes. After two or three weaks of incubation, a very rich growth of chytridiaceous fungi was observed. The developing zoosporic fungi were isolated by using the simple routine methods of investigation of zoosporic fungi (10 round small fragments of the baiting substrata were put into each dish containing water samples) as mentioned by Batko (1975), then examined by using an optical microscope in a bright field in fresh stained conditions, at 20 and 40 x objective lens and then drawing under x 600 and x 800 magnifications with a camera lucida and identified according to Sparrow (1960), Batko (1975) and Karling (1977). Isolates of zoosporic fungi were subcultured on the same substrate by using the induction medium of Mendoza & Prendas (1988). Three times of purifications were used for each isolated species, then maintained on the same medium at 5°C and subcultured every one month.

RESULTS

Fifty-four times of zoosporic fungal isolates belonging to eighteen species of cladochytridiaceous fungi were recorded from eight sites as follows: Maghagha 8, Beni-Mazar 6, Mattai 8, Samalout 11, El-Minia 9, Abo-Qurqas 4, Mallawi 5 and Der-Mawas 3 species; they comprising 44%, 33%, 44%, 61%, 50%, 22%, 28% and 17% of total isolated species, respectively. Samalout was the richest site which contains 11 species representing 61% of total isolated species (11 out of 18). However, the poorest site was Der-Mawas containing 3 species comprising 17% of total number of isolated species.

Nowakowskiella ramosa was the most frequent species in all sites. *N. hemisphaerospora* was observed in the present study as the second most frequent cladochytridiaceous fungus recorded in all sites except Der-Mawas.

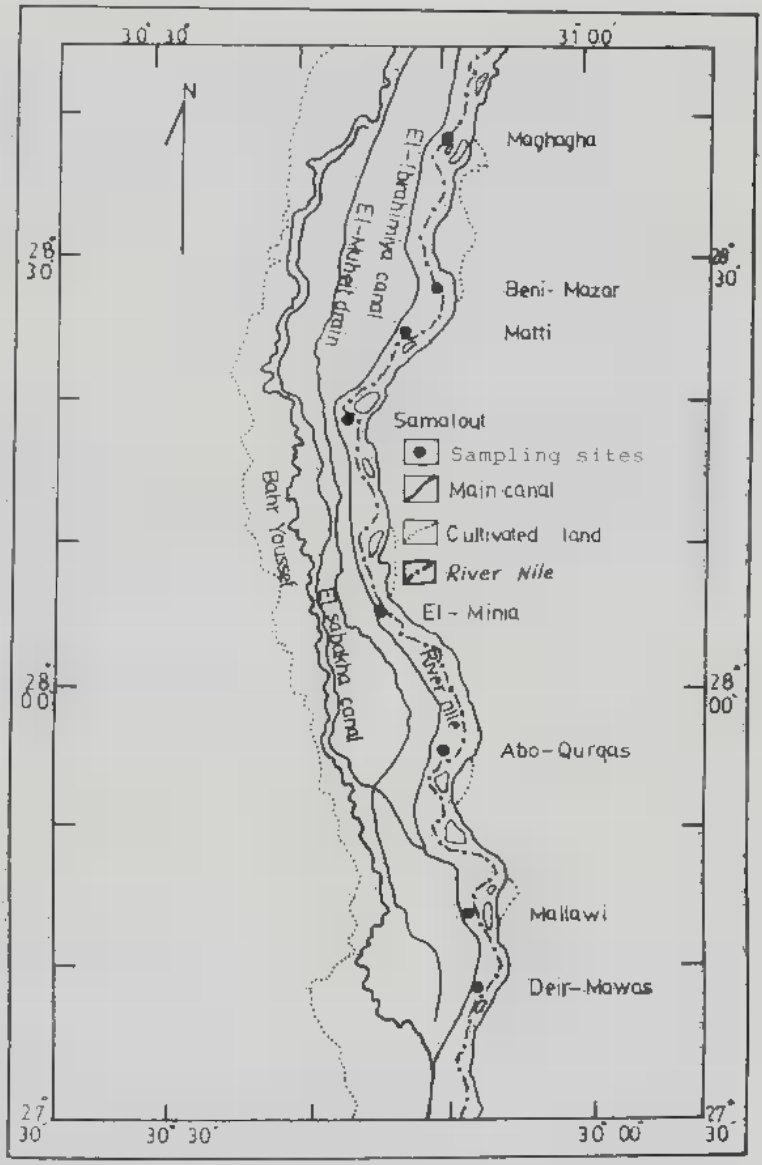

Fig. 1 - Map showing the location of samples collected in El-Minia Governorate.

A) Species recorded for the first time in Egypt:

1 - *Cladochytrium taianum* Shen & Siang 1948 (Plate 1).

Rhizomycelium intramatrical and extramatrical, 0.5-3μm wide, branched, often anastomosing, with numerous fusiform swellings 7-10 x

4-8μm; zoosporangia extramatrical, terminal or intercalary, inoperculate, variable in shape and size, globose, subglobose or ovoid, 21-40μm diam.; zoospores hyaline, globose to subglobose, 11μm diam., containing a single refractive body, 6μm diam., posteriorly uniflagellate; resting spores terminal or intercalary, mostly globose, 17-20μm diam., with a single central oil globule, 8μm diam., wall smooth, about 0.8μm thick.

The Egyptian isolate of *C. taianum* matches the original description in all respects except the resting spores. They appear with thicker wall and intercalary occurrence only.

2 - *Nowakowskiella crassa* Karling 1949 (Plate 2).

Rhizoidal system hyaline, profusely branched, usually wide and thick-walled, with numerous nonseptate, subglobose, fusiform swellings, 9-22 x 6-12μm; zoosporangia intramatrical ovoid, terminal, smooth, globose or oval, 22-45 x 16-40μm, operculum 6-9μm diam.; zoospores globose to subglobose, 5-5.5μm diam., with a single refractive globule 1.5μm diam., with a posterior flagellum; resting spores hyaline, smooth, globose, oval, 20-28 x 16-26μm.

The present values of *N. crassa* closely fit the original ones. Resting spores were observed for the first time.

3 - *Nowakowskiella pitcairnensis* Karling 1968 (Plate 3).

Rhizomycelium profuse, richly branched, tenuous portions 1.5-3.9μm diam., bearing numerous non-septate swellings, narrowly ovoid, fusiform, 8-15 x 4-11μm. Zoosporangia usually terminal, sometimes intercalary, non-apophysate, hyaline, smooth, globose, ovoid, 13-28 x 9-17μm; zoospores, globose, 3-3.3μm diam., with a minute hyaline refractive globule. Resting spores usually abundant, formed from intercalary swelling, fairly thick-walled, light brown filled with coarse granules, smooth, almost globose, 9-10μm diam.

The Egyptian isolate of *N. pitcairnensis* matches the original description by Karling (1968).

4 - *Nowakowskiella macrospora* Karling 1945 (Plate 4).

Rhizomycelium profuse, richly-branched, fairly coarse, tenuous portions 1.6-6.2μm diam.; sporangia extramatrical, terminal or intercalary, hyaline, smooth, usually apophysate, often slightly flattened, globose, oval, 22-32 x 16-24μm. Zoospores slowly oozing out and forming a globular mass at the orifice, amoeboid shape and mostly globose 10.5μm diam., with a large (3-4.6μm), somewhat disc-shaped refractive globule, and numerous minute granules at the posterior end; flagellum 34-42μm long; resting spores globose, 8.5-9.2μm diam., functioning as germinating prosporangia.

Except for the smaller size of resting spores, all other features of the Egyptian isolate match those of the species.

5 - *Megachytrium westonii* Sparrow 1933 (Plate 5).

Thallus at first entirely extramatrical, later also intramatrical consisting of a profusely branched extensive tubular undulating hypha-like vegetative system, whose main axes are up to 6-8.5μm wide, the smaller ones about 2.5μm, and of numerous terminal or intercalary swelling; sporangia globose or subglobose, with or without a single short exit tube, varing greatly in size, usually about 27-39 x 20-34μm, sometimes apophysate, rarely proliferating, wall slightly thick, smooth, colourless; zoospores globose, 5μm diam., with a small colourless globule and a 35μm long flagellum; resting spores broadly ovoid, 20 x 18μm, with a thick smooth wall, filled with globules.

6 - *Septochytrium macrosporum* Karling 1942 (Plate 6).

Thallus predominantly polycentric, occasionally monocentric. Rhizomycelium usually wide, thick-walled, richly branched, rarely septate, tenuous apart from 2-13μm wide rhizoids; rhizoids numerous and richly branched; intercalary swellings broadly or narrowly spindle, elongate, fusiform and irregular. Zoosporangia terminal or intercalary, delimited by septa, globose, pyriform and sometimes irregular, 25-70 x 24-41μm, hyaline and smooth. Zoospores globose up to 11μm diam., when fully formed, remaining a few moments in a globular mass at the orifice before swimming away; intermittently amoeboid. Resting spores intercalary, subglobose, 25-30μm diam., with several large refractive globules.

The rhizomycelium and the zoospores of the Egyptian isolate exactly match the original description.

7 - *Septochytrium marylandicum* Karling 1951 (Plate 7).

Rhizomycelium profuse, much branched, rarely septate up to 5μm diam., with broadly or narrowly fusiform or variously shaped intercalary swellings, bearing slender rhizoids. Sporangia predominantly oval, broadly pyriform, sometimes globose 18-45 x 16-42μm, rarely apophysate, usually with the long axis perpendicular to the rhizoidal axis; discharge tube long, curved, coiled or contorted, simple or branched, occasionally with operculum slightly sunken 5-13μm diam.; zoospores globose, 4.5-5 μm diam., with numerous minute refractive granules and up to 32μm long flagellum; resting spores usually terminal, globose up to 36μm diam., with surround hairs, slightly thick-walled, with numerous small oil bodies.

The Egyptian isolate of *S. marylandicum* closely matches the original description. Resting spores are here recorded for the first time.

B) Species recorded several times in the present study:

1 - *Cladochytrium tenue* Nowakowski 1876.
[recorded 2 times, representing 3.7% (2 out of 54)]

2 - *Cladochytrium aurantiacum* Richards 1956.
[recorded 4 times, representing 7.4% (4 out of 54)].

3 - *Cladochytrium crassum* Hillegas 1941.
[recorded 2 times, representing 3.7% (2 out of 54)].

4 - *Cladochytrium hyalinum* Berdan 1941.
[recorded 5 times, epresenting 9.3% (5 out of 54)].

5 - *Nowakowskiella delia* Whiffen 1943.
[recorded 3 times, representing 5.6% (3 out of 54)].

6 - *Nowakowskiella elongata* Karling 1944.
[recorded 5 times, representing 9.3% (5 out of 54)].

7 - *Nowakowskiella granulata* Karling 1944.
[recorded 4 times, representing 7.4% (4 out of 54)].

8 - *Nowakowskiella hemisphaerospora* Shanor 1942.
[recorded 7 times, representing 13% (7 out of 54)].

9 - *Nowakowskiella multispora* Karling 1964.
[recorded 2 times, representing 3.7% (2 out of 54)].

10 - *Nowakowskiella ramosa* Butler 1907.
[recorded 8 times, representing 14.8% (8 out of 54)].

11 - *Septochytrium variabile* Berdan 1939.
[recorded 5 times, representing 9.3% (5 out of 54)].

DISCUSSION

The Cladochytriaceae now comprise 33 species in 7 genera all over the world. In the present study, 18 species belonging to four genera (*Nowakowskiella* 9, *Cladochytrium* 5, *Septochytrium* 3, *Megachytrium* 1) have been found. Seven were observed here for the first time in Egypt. *Cladochytrium tenue* was recorded in Egypt for the second time.

Nowakowskiella represents 50% of total number of the species isolated in this study. Of sixteen species found in different parts of the world (Batko & Hassan, 1982; Hassan & Batko, 1986), over half are now known from Egypt. *Nowakowskiella ramosa*, *N. hemisphaerospora* and *N. macrospora* are the most common and widely distributed species (Sparrow, 1960; Karling, 1968, 1977; Hassan, 1982; Elnaghy et al., 1985b). Karling (1945) clearly pointed out that in *N. macrospora* the typical mode of zoospore discharge is by extrusion of the slightly sunken operculum. This agrees exactly with my own observations. Although Shen & Siang (1948) mentioned an operculum, they did not indicate whether it is a true operculum or an endo-operculum. Most *Nowakowskiella* species are exo-operculate except for *N. granulata* and *N. macrospora* which are endo-operculate. Zoosporangia of *N. multispora* are endo- and exo-operculate.

Twelve species of *Cladochytrium* have been recorded from all over the world (Karling, 1977; Batko & Hassan, 1986). Over 40% of the known species are reported in this study. *C. tenue* and *C. hyalinum* are the most common and widely distributed in water (Sparrow, 1960; Batko, 1975; Hassan, 1982). Striking features of *C. hyalinum* are large oil globule in the zoospores and a strong tendency for extramatrical development. On germination, zoospores of *C. tenue* produce one or two delicate filaments,

which after a few days become elongate and irregularly branched. Resting spores were observed in *C. crassum* after a long time, as described by Karling (1945) and Elnaghy et al. (1987). *C. taianum* is distinguished from *C. tenue* by larger zoospores.

Septochytrium Berdan represents 16.7% of the total number of species isolated in this investigation (3 out of 18). At present, five species are known, three of them were found in this study. Willoughby (1964) found that a part of zoospores of *S. marylandicum* develop into a primary zoosporangium and rhizoidal axes. A similar sequence of events has also been observed occasionally in *S. macrosporum*. *S. macrosporum* differs from *S. marylandicum* by its smaller zoospores (4.5-5μm and 10.8-11.2μm diam., respectively).

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Plate 1, Figs. 1-8: *Cladochytium taianum*. 1: Zoospores with refractive globule; 2-6: Young terminal and intercalary zoosporangia; 7: Portion of rhizomycelium showing young sporangia and spindle organs; 8: Resting spores with rhizomycelium.

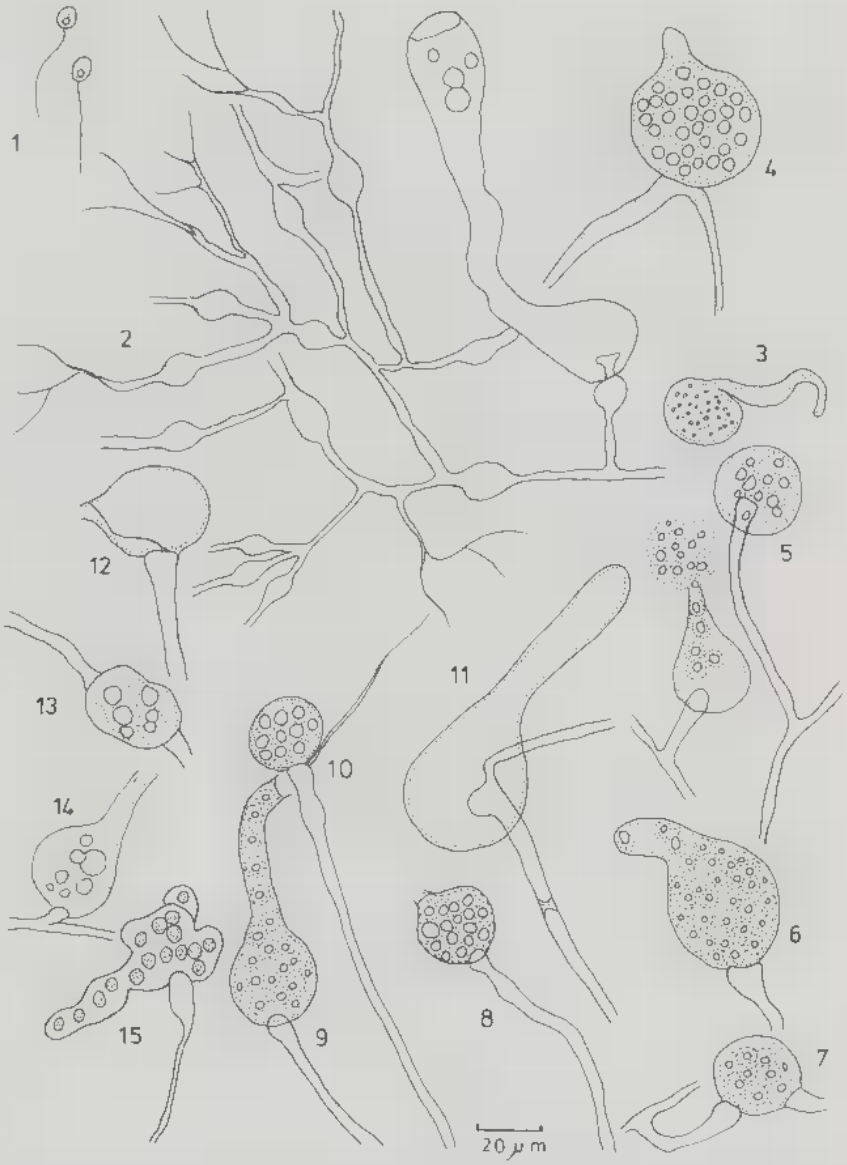

Plate 2, Figs. 1-15: *Nowakowskiella crassa*. 1: Zoospores with refractive globule; 2: Portion of rhizomycelium showing spindle organs, elongate sporangium and tapering rhizoidal ends; 3: Zoosporangial germination; 4-10: Different shapes of young sporangia; 11, 12: Empty zoosporangia; 13-15: Resting spores with various shapes.

Plate 3, Figs. 1-5: *Nowakowskiella pitcairnensis.* 1: Zoospores with refractive globule; 2, 3: Extensive growth of rhizomycelium showing numerous spindle organs and tenuous rhizoidal system; 4: Portion of rhizomycelium with young zoosporangia; 5: Resting spores with rhizomycelium.

Plate 4, Figs. 1-4: *Nowakowskiella macrospora*. 1: Differently shaped zoospores; 2: Portion of polycentric rhizomycelium with young zoosporangia; 3: Part of rhizomycelium showing operculate sporangium and rhizoidal system; 4: Another portion of rhizomycelium with young sporangium and germinating resting spore.

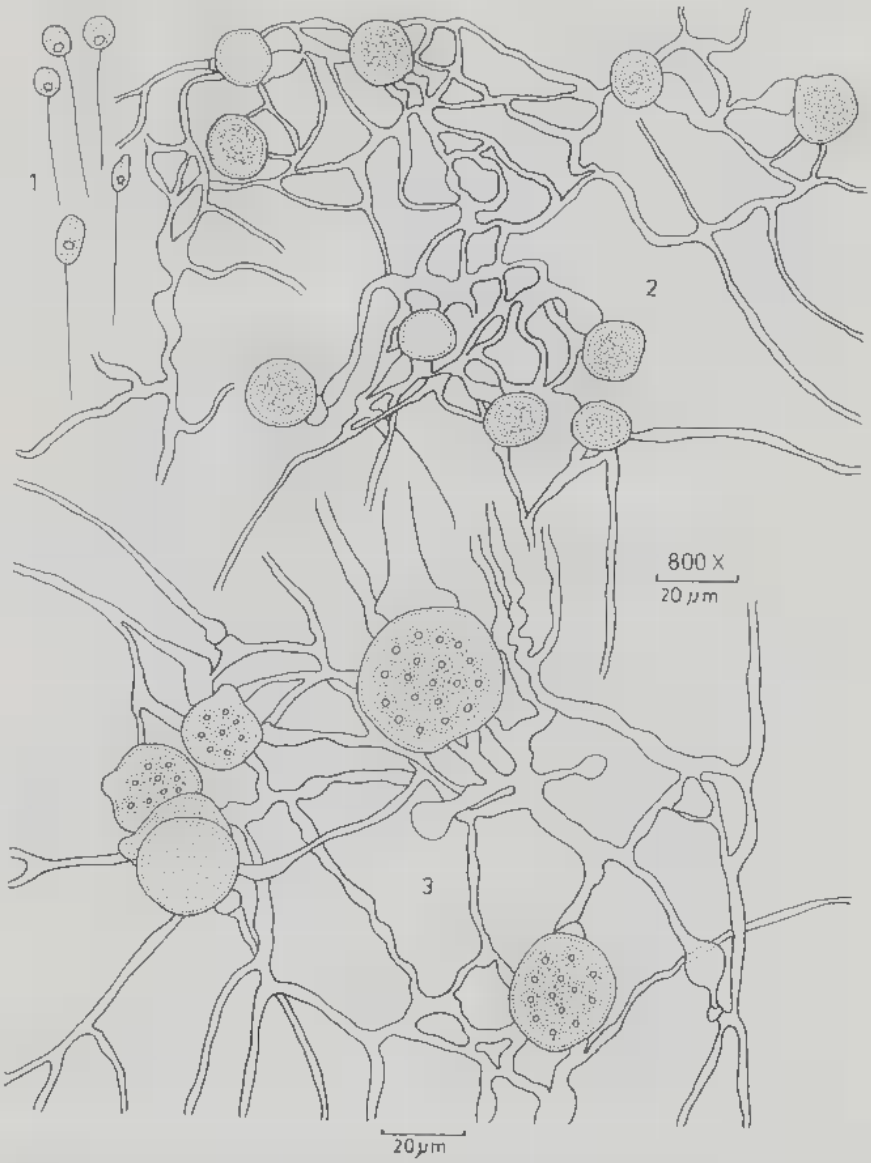

Plate 5, Figs. 1-3: *Megachytrium westonii*. 1: Zoospores with accentric refractive globule; 2: Portion of polycentric rhizomycelium showing terminal and intercalary zoosporangia and swelling bodies; 3: Extensive growth of rhizomycelium with young and mature sporangia and resting spores.

Plate 6, Figs. 1-3: *Sepochytrium macrosporum*. 1: Zoospores; 2: Extensive growth of rhizomycelium showing different shapes of zoosporangia, spindle organs and some septa; 3: Operculate sporangium.

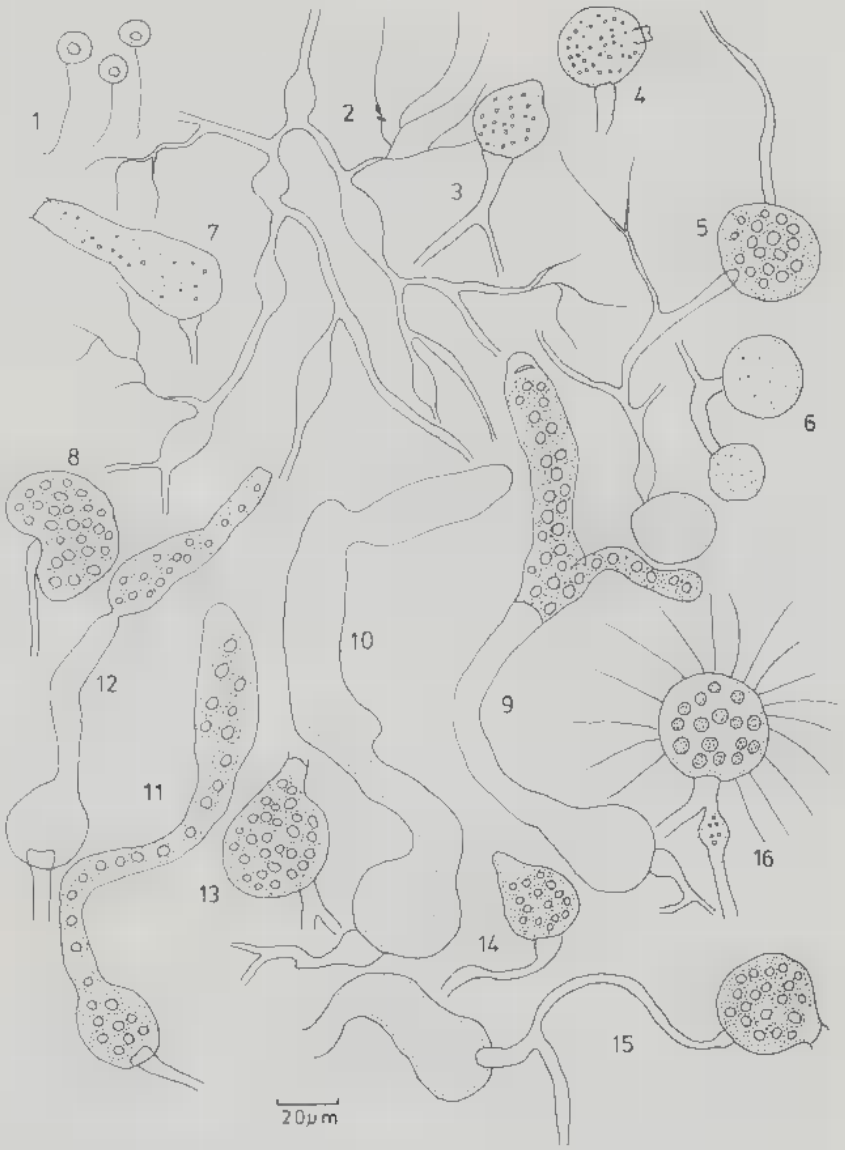

Plate 7, Figs. 1-16: *Septochytrium marylandicum*. 1: Zoospores; 2: Portion of rhizomycelium with spindle organs and tenuous rhizoidal system; 3, 4, 6-8, 13-15: Variously shaped young and mature terminal sporangia; 5: Rhizomycelium with intercalary mature sporangium and terminal young one; 9-12: Four elongate zoosporangia with 1-2 exit tubes; 16: Slightly thick-walled resting spore with hairs.